

**Amendments to the Claims:**

This listing of claims will replace all prior versions, and listings, of claims in the application:

**Listing of Claims:**

1. (Withdrawn) A microfluidic device adapted for conducting assays comprising a solid substrate layer having a surface that is capable of attaching ligand and/or anti-ligand, and an elastomeric layer attached to said solid substrate surface, wherein said elastomeric layer comprises:

(a) a plurality of first flow channels;

(b) a plurality of second flow channels each intersecting and crossing each of said first flow channels thereby providing a plurality of intersecting areas formed at intersections between said first flow channels and said second flow channels, wherein said plurality of first flow channels and said plurality of second flow channels are adapted to allow the flow of a solution therethrough, and wherein said solid substrate surface is in fluid communication with at least said intersecting areas of said plurality of first flow channels and said plurality of second flow channels, and wherein said plurality of first flow channels and/or said plurality of second flow channels are capable of forming a plurality of looped flow channels;

(c) a plurality of control channels;

(d) a plurality of first control valves each operatively disposed with respect to each of said first flow channel to regulate flow of the solution through said first flow channels, wherein each of said first control valves comprises a first control channel and an elastomeric segment that is deflectable into or retractable from said first flow channel upon which said first control valve operates in response to an actuation force applied to said first control channel, the elastomeric segment when positioned in said first flow channel restricting solution flow therethrough;

(e) a plurality of second control valves each operatively disposed with respect to each of said second flow channel to regulate flow of the solution through said second flow

channels, wherein each of said second control valves comprises a second control channel and an elastomeric segment that is deflectable into or retractable from said second flow channel upon which said second control valve operates in response to an actuation force applied to said second control channel, the elastomeric segment when positioned in said second flow channel restricting solution flow therethrough;

(f) a plurality of loop forming control valves each operatively disposed with respect to each of said first and/or said second flow channels to form said plurality of looped flow channels, wherein each of said loop forming control valves comprises a loop forming control channel and an elastomeric segment that is deflectable into or retractable from said first and/or said second flow channels upon which said loop forming control valve operates in response to an actuation force applied to said loop forming control channel, the elastomeric segment when positioned in said first and/or said second flow channels restricting solution flow therethrough thereby forming said looped flow channel; and

(g) a plurality of recirculating pumps, and wherein each recirculating pump is operatively disposed with respect to one of said looped flow channels such that circulation of solution through each of said looped flow channels can be regulated by one of said recirculating pumps.

2. (Withdrawn) The microfluidic device of Claim 1, wherein each of said plurality of recirculating pumps comprises more than one control channels each formed within said elastomeric layer and separated from said looped flow channel by an elastomeric segment which is deflectable into said looped flow channel in response to an actuation force.

3. (Withdrawn) The microfluidic device of Claim 1, wherein actuation of both of said plurality of first control valves and said plurality of second control valves forms a plurality of holding valves each of which is operatively disposed with respect to each of said first and said second flow channels such that a holding space encapsulating each of said intersecting area is formed.

4. (Withdrawn) The microfluidic device of Claim 1 further comprising a solution inlet for each of said first flow channels in fluid communication therewith for introduction of a first solution.

5. (Withdrawn) The microfluidic device of Claim 4 further comprising a second solution inlet for each of said second flow channels in fluid communication therewith for introduction of a second solution.

6. (Withdrawn) The microfluidic device of Claim 1, wherein said plurality of first flow channels and said plurality of second flow channels are located on the interface between said solid substrate layer and said elastomeric layer such that one side of each of said first and said second flow channels is formed by said solid substrate surface.

7. (Withdrawn) The microfluidic device of Claim 1, wherein said plurality of first flow channels and said plurality of second flow channels are located within said elastomeric layer, and wherein each of said plurality of intersecting areas formed at intersections between said first flow channels and said second flow channels comprises a via which is in fluid communication with said solid substrate surface thereby forming a well that is adapted to collect a fluid therein.

8. (Withdrawn) The microfluidic device of Claim 1 further comprising a plurality of first flow channel pumps, wherein each of said first flow channel pump is operatively disposed with respect to one of said first flow channels such that solution flow through each of said first flow channels can be regulated by one of the pumps.

9. (Withdrawn) The microfluidic device of Claim 8 further comprising a plurality of second flow channel pumps, wherein each of said second flow channel pump is operatively disposed with respect to one of said second flow channels such that solution flow through each of said second flow channels can be regulated by one of the pumps.

10. (Withdrawn) The microfluidic device of Claim 9, wherein each of said plurality of flow channel pumps comprises more than one control channels each formed within said elastomeric layer and separated from said flow channel by an elastomeric segment which is deflectable into said flow channel in response to an actuation force.

11. (Withdrawn) The microfluidic device of Claim 1 further comprising a first solution outlet channel in fluid communication with each of said first flow channel such that the solution from each of said first flow channel flow out into said first solution outlet channel.

12. (Withdrawn) The microfluidic device of Claim 1 further comprising a second solution outlet channel in fluid communication with each of said second flow channel such that the solution from each of said second flow channel flow out into said second solution outlet channel.

13. (Withdrawn) The microfluidic device of Claim 1, wherein said solid support surface at each of said intersecting areas comprises a ligand that is capable of specifically binding to antiligand that are present in the solution.

14. (Previously Presented) A method of conducting a binding assay, comprising:

(a) providing a microfluidic device comprising a solid substrate layer having a surface that is capable of attaching ligand and/or anti-ligand, and an elastomeric layer attached to said solid substrate surface, wherein said elastomeric layer comprises:

(i) a plurality of first flow channels;

(ii) a plurality of second flow channels each intersecting and crossing each of the first flow channels thereby providing a plurality of intersecting areas formed at intersections between the first flow channels and the second flow channels, wherein the plurality of first flow channels and the plurality of second flow channels are adapted to allow the flow of a solution therethrough, wherein the solid substrate surface is in fluid communication with at least the intersecting areas of the plurality of first flow channels and the plurality of second flow channels,

and wherein the plurality of first flow channels and/or the plurality of second flow channels are capable of forming a plurality of closed looped flow channels;

(iii) a plurality of control channels;

(iv) a plurality of first control valves each operatively disposed with respect to each of the first flow channels to regulate flow of the solution through the first flow channels, wherein each of the first control valves comprises a first control channel and an elastomeric segment that is deflectable into or retractable from the first flow channel upon which the first control valve operates in response to an actuation force applied to the first control channel, the elastomeric segment when positioned in the first flow channel restricting solution flow therethrough;

(v) a plurality of second control valves each operatively disposed with respect to each of the second flow channels to regulate flow of the solution through the second flow channels, wherein each of the second control valves comprises a second control channel and an elastomeric segment that is deflectable into or retractable from the second flow channel upon which the second control valve operates in response to an actuation force applied to the second control channel, the elastomeric segment when positioned in the second flow channel restricting solution flow therethrough;

(vi) a plurality of sets of closed loop forming control valves each set having a first valve operatively disposed with respect to an inlet of one of each of the first and/or the second flow channels and a second valve operatively disposed with respect to an outlet of one of each of the first and/or second flow channels to form the plurality of closed looped flow channels, wherein each of the loop forming control valves comprises a loop forming control channel and an elastomeric segment that is deflectable into or retractable from the first and/or the second flow channels upon which the loop forming control valve operates in response to an actuation force applied to the loop forming control channel, the elastomeric segment when positioned in the first and/or the second flow channels restricting solution flow therethrough thereby forming the looped flow channel; and

(vii) a plurality of recirculating pumps, and wherein each recirculating pump is operatively disposed with respect to one of the closed looped flow channels such that circulation of solution through each of the closed looped flow channels can be regulated by one of the recirculating pumps;

(b) applying an actuating force to the second control valves to restrict solution flow through each of the second flow channels;

(c) introducing a reagent comprising a ligand into at least one of the first flow channels under conditions sufficient to attach the ligand to the solid substrate surface;

(d) removing the actuation force to the second flow channel control channel and applying an actuation force to the first control channel such that solution flow through the first flow channel is restricted; and

(e) performing a binding assay by introducing a sample solution into the second flow channel;

(f) applying an actuating force from two control lines to the plurality of sets of closed loop forming control valves to form the plurality of closed looped flow channels such that each closed looped flow channel comprises a closed loop spanning multiple rows and multiple columns; and recirculating the sample solution within the closed loop each of the closed looped flow channels using the recirculating pump under conditions sufficient to specifically bind an anti-ligand that may be present in the sample solution to the ligand that is attached to the solid substrate surface; and

(g) detecting the binding of the anti-ligand in the sample to the ligand.

15. (Original) The method of Claim 14 further comprising removing any ligand that is not attached to the solid substrate surface from the first flow channel prior to introducing the sample solution into the second flow channel.

16. - 17. (Canceled)

18. (Original) The method of Claim 14, wherein the plurality of first flow channels and the plurality of second flow channels are located within the elastomeric layer, and wherein each of the intersecting areas formed at intersections between the first flow channels and the second flow channels comprises a via which is in fluid communication with the solid substrate surface thereby forming a well that is adapted to collect a fluid therein.

19. (Original) The method of Claim 14, wherein the first flow channel is in communication with a pump, and wherein the reagent is transported through the first flow channel under the action of the pump.

20. (Original) The method of Claim 19, wherein the pump comprises more than one control channels each formed within the elastomeric layer and separated from the first flow channel by an elastomeric segment that is deflectable into the first flow channel in response to an actuation force, whereby the reagent is transported along the first flow channel.

21. (Original) The method of Claim 14, wherein the second flow channel is in communication with a pump, and wherein the sample solution is transported through the second flow channel under the action of the pump.

22. (Previously Presented) The method of Claim 21, wherein the pump comprises more than one control channels each formed within the elastomeric layer and separated from the second flow channel by an elastomeric segment that is deflectable into the second flow channel in response to an actuation force, whereby the sample solution is transported along the second flow channel upon independent, sequential actuation of the more than one control channels.

23. (Original) The method of Claim 14, wherein said step (e) of performing binding assay comprises removing the elastomeric layer from the solid substrate surface and determining ligand/antiligand binding at each of the intersecting areas with a detector.

24. (Original) The method of Claim 23, wherein the detector detects an optical signal within the intersecting areas.

25. (Original) The method of Claim 24, wherein the detector detects a fluorescence emission, fluorescence polarization or fluorescence resonance energy transfer.

26. (Original) The method of Claim 24, wherein the detector is an optical microscope, a confocal microscope or a laser scanning confocal microscope.

27. (Original) The method of Claim 23, wherein the detector is a non-optical sensor selected from the group consisting of a radioactivity sensor, and an electrical potential difference sensor.

28. (Previously Presented) The method of Claim 14, wherein the assay comprises detecting binding between a substrate and a cell receptor; a substrate and an enzyme; an antibody and an antigen; a nucleic acid and a nucleic acid binding protein; a protein and a protein; a small molecule and a protein; a small molecule and an oligonucleotide; or a protein affinity tag and a metal ion.

29. (Original) The method of Claim 14, wherein the assay is an assay for detecting a toxic effect on cells or a cell death assay, or a cell proliferation assay.

30. (Original) The method of Claim 14, wherein the assay is an oligonucleotide binding assay or a peptide binding assay.

31. (Previously Presented) The method of Claim 14, wherein the assay is an antimicrobial assay.

32. (Withdrawn) A method for producing a microfluidic device comprising:  
(a) producing a control layer, a flow layer, and a via layer from an elastomeric polymer, wherein each of the control layer and the flow layer comprises grooves on its surface for forming control channels and flow channels, respectively;



(b) attaching the control layer to the flow layer such that the grooves in the control layer is attached to a top surface of the flow layer thereby forming a plurality of control channels and attaching the bottom surface of the flow layer to the via layer thereby forming a plurality of first flow channels and a plurality of second flow channels, wherein each first flow channels intersects and crosses each of the second flow channels thereby forming a plurality of channel intersections, and wherein each vias in the via layer is positioned at each channel intersections; and

(c) optionally attaching the elastomeric polymer produced in said step (b) to a solid substrate which is comprises a ligand bound to its surface or comprises a functional group which is capable of attaching a ligand.

33. (Withdrawn) The method of Claim 32, wherein said step of producing the via layer further comprises etching the via layer to produce a plurality of vias.

34. (Previously Presented) A method of conducting a binding assay, comprising:

(a) providing a microfluidic device comprising a solid substrate layer having a surface that is capable of attaching ligand and/or anti-ligand, and an elastomeric layer attached to said solid substrate surface, wherein said elastomeric layer comprises:

(i) a first flow channel;

(ii) a second flow channel intersecting and crossing the first flow channel thereby providing an intersecting area formed at an intersection between the first flow channel and the second flow channel, wherein the first flow channel and the second flow channel are adapted to allow the flow of a solution therethrough, wherein the solid substrate surface is in fluid communication with at least the intersecting area of the first flow channel and the second flow channel, and wherein the first flow channel and/or the second flow channel are capable of forming a closed looped flow channel;

(iii) a plurality of control channels;

(iv) a first control valve operatively disposed with respect to the first flow channel to regulate flow of the solution through the first flow channel, wherein the first control valve comprises a first control channel and an elastomeric segment that is deflectable into or retractable from the first flow channel upon which the first control valve operates in response to an actuation force applied to the first control channel, the elastomeric segment when positioned in the first flow channel restricting solution flow therethrough;

(v) a second control valve operatively disposed with respect to the second flow channel to regulate flow of the solution through the second flow channel, wherein the second control valve comprises a second control channel and an elastomeric segment that is deflectable into or retractable from the second flow channel upon which the second control valve operates in response to an actuation force applied to the second control channel, the elastomeric segment when positioned in the second flow channel restricting solution flow therethrough;

(vi) a first loop forming control valve operatively disposed with respect to an inlet of one of the first and/or the second flow channels and a second loop forming control valve operatively disposed with respect to an outlet of one of the first and/or second flow channels to form the closed looped flow channel, wherein each of the first and second loop forming control valves comprises a loop forming control channel and an elastomeric segment that is deflectable into or retractable from the first and/or the second flow channels upon which the loop forming control valve operates in response to an actuation force applied to the loop forming control channel, the elastomeric segment when positioned in the first and/or the second flow channels restricting solution flow therethrough thereby forming the closed looped flow channel; and

(vii) a recirculating pump operatively disposed with respect to the closed looped flow channel such that circulation of solution through the closed looped flow channel can be regulated by the recirculating pump;

(b) applying an actuating force to the second control valve to restrict solution flow through the second flow channel;

(c) introducing a reagent comprising a ligand into the first flow channel under conditions sufficient to attach the ligand to the solid substrate surface;

(d) removing the actuation force to the second flow channel control channel and applying an actuation force to the first control channel such that solution flow through the first flow channel is restricted; and

(e) performing a binding assay by introducing a sample solution into the second flow channel;

(f) applying an actuating force to the first loop forming control valve using a first control line and to the second loop forming control valve using a second control line to form the closed looped flow channel such that the closed looped flow channel comprises a closed loop; and recirculating the sample solution within the closed loop of the closed looped flow channel using the recirculating pump under conditions sufficient to specifically bind an anti-ligand that may be present in the sample solution to the ligand that is attached to the solid substrate surface; and

(g) detecting the binding of the anti-ligand in the sample to the ligand.

35. (Previously Presented) The method of Claim 34, wherein the first flow channel and the second flow channel are located within the elastomeric layer, and wherein the intersecting area formed at the intersection between the first flow channel and the second flow channel comprises a via which is in fluid communication with the solid substrate surface thereby forming a well that is adapted to collect a fluid therein.

36. (Previously Presented) The method of Claim 34, wherein said step (e) of performing binding assay comprises removing the elastomeric layer from the solid substrate surface and determining ligand/antiligand binding at the intersecting area with a detector, wherein the detector detects an optical signal within the intersecting areas, and wherein the detector detects a fluorescence emission, fluorescence polarization, or fluorescence resonance energy transfer.

37. (Previously Presented) The method of Claim 34, wherein said step (e) of performing binding assay comprises removing the elastomeric layer from the solid substrate

surface and determining ligand/antiligand binding at the intersecting area with a detector, wherein the detector detects an optical signal within the intersecting areas, and wherein the detector is an optical microscope, a confocal microscope, or a laser scanning confocal microscope.

38. (Previously Presented) The method of Claim 22, wherein the first valve of the set of loop forming control valves comprises a control channel of the pump that is independently actuated with respect to other control channels of the pump.

39. (Previously Presented) The method of Claim 34, wherein the second flow channel is in communication with a pump comprising a plurality of sequentially actuated control channels, and wherein the sample solution is transported through the second flow channel under the action of the pump.

40. (Previously Presented) The method of Claim 39, wherein the first loop forming control valve comprises a control channel of the pump.